1: Mol Microbiol. 1995 Jan;15(2):277-85.

Construction of recombinant neisserial Hsp60 proteins and mapping of antigenic domains.

Pannekoek Y, Dankert J, van Putten JP.

Max-Planck-Institut fur Biologie, Abteilung Infektionsbiologie, Tubingen, Germany.

Here we report the cloning and expression, in Escherichia coli, of PCR-amplified DNA encoding the 63-kDa stress-inducible protein of Neisseria gonorrhoeae strains VP1 and PID2, Neisseria meningitidis 2996 and the commensal Neisseria flavescens. DNA sequence analysis revealed in all cases one open reading frame of 541-544 amino acids corresponding to a protein of approximately 57,000 Da. The various neisserial proteins were > 96% identical at the amino acid level and showed extensive homology with proteins belonging to the Hsp60 heat-shock-protein family. We constructed defined glutathione S-transferase fusion polypeptides of the gonococcal Hsp60 homologue to locate antigenic domains on the recombinant protein. Variation in the immunoreactivity of two monoclonal antibodies recognizing a conserved and a neisseria-unique antigenic Hsp60 determinant, respectively, could thus be deduced to result from single amino acid substitutions. Analysis of the antibody response in patients' sera demonstrated reactivity with the same fusion polypeptides in six out of nine sera, indicating that neisserial Hsp60 is expressed during the natural infection and that distinct domains on the protein are immunodominant in vivo.

PMID: 7746149 [PubMed - indexed for MEDLINE]

ELSEVIER

FEMS Immunology and Medical Microbiology 26 (1999) 209–226

FEMS IMMUNOLOGY AND MEDICAL MICROBIOLOGY

Molecular mimetics of polysaccharide epitopes as vaccine candidates for prevention of *Neisseria meningitidis* serogroup B disease

Gregory R. Moe, Siqi Tan, Dan M. Granoff *

Children's Hospital Oakland Research Institute, 747 52nd Street, Oakland, CA 94609, USA

Received 8 March 1999; revised 14 July 1999; accepted 21 July 1999

Abstract

Neisseria meningitidis is a major cause of meningitis and sepsis. Despite nearly 25 years of work, there is no promising vaccine candidate for prevention of disease caused by meningococcal B strains. This review summarizes newer approaches for eliciting protective meningococcal B immune responses, including the use of molecular mimetics of group B polysaccharide and conserved membrane proteins as immunogens. The capsular polysaccharide of this organism is conserved and serum antibody to this capsule confers protection against disease. However, the immunogenicity of meningococcal B polysaccharide-based vaccines is poor. Further, a portion of the antibody elicited has autoantibody activity. Recently, our laboratory produced a panel of murine monoclonal antibodies (Mabs) that react specifically with capsular polysaccharide epitopes on meningococcal B that are distinct from host polysialic acid. These Mabs elicit complement-mediated bactericidal activity and confer passive protection in animal models. The anti-capsular Mabs were used to identify molecular mimetics from phage display peptide libraries. The resulting peptides were antigenic mimetics as defined by binding to the Mabs used to select them but, to date, are poor immunogenic mimetics in failing to elicit anti-capsular antibodies.

Keywords: Meningococcal disease; Vaccine; Molecular mimetic; Anti-idiotype; Peptide mimetic; Phage display

1. Introduction

The disease burden caused by *Neisseria meningitidis* is considerable. With the control of *Haemophilus influenzae* type b disease by vaccination, *N. meningitidis* has emerged as one of the most common causes of meningitis in children and young adults. Despite the availability of anti-microbial therapy, case fatality rates from meningococcal meningitis average 5–10% [1–5] and up to 25% of survivors are left with neurosensory hearing loss or other neurological sequelae [5]. This organism is also an important cause of sepsis and endotoxin shock (case fatality rates up to 40%) [1–5].

Strains of *N. meningitidis* can be divided into 12 serogroups based on chemically and antigenically distinctive polysaccharide capsules [6]. Five serogroups, designated A, B, C, Y and W-135, account

* Corresponding author. Tel.: +1 (510) 450-7640; Fax: +1 (510) 428-3608; E-mail: dgranoff@chori.org

0928-8244/99/$20.00
PII: S0928-8244(99)00144-3

for virtually all disease-producing isolates. In Europe, group B isolates predominate (50–80% of isolates), with most of the remaining isolates being serogroup C [2,4,7].

N. meningitidis also has a propensity to cause epidemics. Cyclic epidemics of serogroup A disease occur every 5–10 years in sub-Saharan countries of Africa [8,9]. These epidemics can result in hundreds of thousands of cases in a single season, with tens of thousands of deaths. Outbreaks of serogroup C disease also have emerged as an important clinical problem in North America [11,12] and serogroup B epidemics have occurred periodically in Europe, the Americas and in New Zealand [3,10,13,14]. Given this large disease burden in the developed and developing world, an effective vaccine for prevention of *N. meningitidis* disease remains an important public health priority.

First generation, plain polysaccharide vaccines are available for prevention of meningococcal disease caused by serogroups A, C, Y and W-135 [15–17]. These vaccines are safe and in older children and adults induce complement-mediated bactericidal antibody that confers protection against disease [15,17]. However, the plain polysaccharide vaccines are poorly immunogenic in infants and young children and, thus, fail to confer protection to the age groups with the highest incidence of meningococcal disease [15]. Several commercial organizations are developing meningococcal polysaccharide-protein conjugate vaccines against *N. meningitidis* strains of serogroups A and C or A, C, Y and W-135. These conjugate vaccines are safe and more immunogenic in infants and toddlers than the plain polysaccharide vaccines [17–21]. The conjugate vaccines also induce long-term immunologic B-cell memory to the plain polysaccharide [19,20], which could be important in protection in immunized individuals with low serum antibody concentrations. Clinical development of several meningococcal conjugate vaccines is very advanced. It, therefore, seems highly likely that a monovalent meningococcal C conjugate vaccine will be introduced for routine immunization in Europe and North America within a few years and that a multivalent vaccine for prevention of A, C, Y and W-135 strains will follow. However, as described below, despite nearly 25 years of work, there is no promising vaccine candidate for prevention of disease caused by meningococcal B strains, which are responsible for up to 80% of meningococcal cases in industrialized countries. The lack of a suitable group B vaccine will, therefore, be an important limitation in the control of meningococcal disease by the new conjugate vaccines [15,17].

A number of experimental approaches have been used to develop vaccines for the prevention of meningococcal B disease. These include vaccines based on eliciting antibody to the group B capsule [22,23] or to non-capsular antigens. The non-capsular approaches include outer membrane vesicle (OMV) vaccines [24–27], specific outer membrane proteins (OMPs) such as PorA [28–34], iron-regulated proteins [35–37], class 4 (Rmp) proteins [38], Opc [39], Neisserial surface protein A [40,41] and detoxified lipopolysaccharide [42–46]. In this minireview, we summarize the status of some of the more promising and/or clinically advanced approaches for development of a meningococcal B vaccine. We also present a summary of data from our laboratory investigating the use of molecular mimetics of the group B polysaccharide as vaccine candidates.

2. Non-capsular antigens of *N. meningitidis* as vaccine candidates for prevention of group B disease

2.1. OMP vesicle-based vaccines

Considerable evidence indicates that certain non-capsular antigens can elicit protective antibody responses (reviewed in [47]). In older children and adults, OMV vaccines have proven to be modestly effective in preventing epidemic meningococcal B disease caused predominantly by a single serosubtype strain [25,48,49]. However, these vaccines did not confer protection in children less than 2–3 years of age, the group at greatest risk of developing meningococcal disease. Also, for reasons described below, the vesicle vaccines provide minimal or no cross-protection against meningococcal B strains with heterologous serosubtypes.

The bactericidal antibody responses to OMV vaccines appear to be directed predominantly at the class 1 porin protein (PorA) [34,50]. The predicted structure of PorA contains eight hydrophilic exposed loops that are created as the porin traverses the outer

membrane (OM) [51,52]. Amino acid variability between different PorA proteins is restricted to specific regions of loops 1 and 4. Interestingly, the bactericidal antibody responses to the vesicle vaccines tend to be directed primarily to these variable regions, which account for the serosubtype specificity of the vaccine-induced immunity [50,52]. Furthermore, even minor sequence variations in a portion of loop 4, designated variable region 2 (VR2), can drastically effect the susceptibility of a strain to complement-mediated bacteriolysis induced by serum antibody elicited by the vesicle vaccine [28].

To expand protection to a greater number of strains, a recombinant hexavalent PorA vesicle vaccine was developed at the Rijksinstituut Voor Volksgezondheid en Milieu (RIVM, The Netherlands) using vesicles prepared from two strains that each express three different PorA proteins [32–34]. The hexavalent PorA OMP vaccine has been evaluated in clinical trials in adults and infants. In most subjects, the bactericidal antibody responses were only modest. Also, compared to those elicited by the corresponding monovalent vesicle vaccine, interference was observed in the immunogenicity between certain serotype antigens contained in the multivalent vaccine [53].

In summary, OMV vaccines can clearly induce protective antibody in humans and for this reason, they have been regarded as important vaccine candidates. However, this approach has some important limitations. These include restricted bactericidal antibody response to hypervariable regions of PorA, the propensity for disease during non-epidemic periods to be caused by multiple strain variants and the potential for antigenic drift that may be accelerated by selective pressures resulting from the immune responses induced by vaccination [54]. Therefore, it will be a formidable challenge to develop a universal meningococcal B vaccine based only on evoking protective antibody responses to the PorA protein or based on other membrane proteins that share the feature of antigenic diversity in the surface-exposed epitopes.

2.2. Transferrin binding protein B (TbpB)

Iron is important in the metabolism of *Neisseria* and this organism has evolved elaborate means to scavenge iron from the environment. TbpB is a subunit of transferrin binding protein, a surface-exposed *N. meningitidis* group B lipoprotein whose expression is up-regulated when the organism is grown in iron-restricted media. Although amino acid sequence variation is common in the B subunit of Tbp [55], recombinant TbpB produced in *Escherichia coli* is immunogenic in experimental animals and elicits high titers of bactericidal antibody that are active against most meningococcal B strains [56,57]. Thus TbpB is an attractive vaccine candidate and is currently being developed by a commercial organization. In a recent phase I study in adult humans, a recombinant TbpB vaccine was reported to elicit high IgG antibody titers as measured by an enzyme-linked immunosorbent assay (ELISA), but the resulting antibodies showed minimal complement-mediated bacteriolytic activity [58]. Given the importance of bactericidal antibody in protection, its absence in most of the post-immunization sera raises concerns about the potential efficacy of this TbpB vaccine in humans. Future vaccine work on TbpB will likely focus on investigating improved formulations and use of adjuvants.

2.3. Neisserial surface protein A (NspA)

NspA was recently described by Martin and Brodeur and coworkers [40]. They generated monoclonal antibodies (Mabs) by sequential immunization of mice with OM preparations from a meningococcal serogroup A strain followed by a meningococcal group C strain. A Mab, designated Me-1, was isolated that was bactericidal against meningococcal A, B and C isolates and cross-reacted with 248 of 250 meningococcal strains in a colony blot ELISA using whole bacterial antigens. This strain collection included representatives of serogroups A, B, C, 29-E, W-135, Y and Z (including 44 serogroup B organisms). Thus, the epitope recognized by Mab Me-1 is highly conserved across *N. meningitidis*. Me-1 is also reported to confer passive protection in mice against a lethal challenge with meningococcal B bacteria. Thus, this epitope is not only present in *Neisseria* organisms grown in vitro but also during infection.

Immunization of mice with recombinant NspA mixed with the adjuvant, QuilA, elicited bactericidal antibody responses against serogroup A, B and C

strains [40]. Immunized mice were also protected from a lethal challenge with live meningococcal B organisms [40]. Recently, recombinant NspA absorbed to alum was also reported to elicit bactericidal antibody responses in rabbits and subhuman primates [41]. However, during natural infection in humans, NspA appears to elicit only weak antibody responses as measured by ELISA in acute- and convalescent-phase sera (generally 2-fold increases or less) [59].

NspA may represent the first of a novel group of promising vaccine candidates that are characterized by being highly conserved *Neisseria* membrane proteins, present in a relatively low copy number in the OM and relatively weak immunogens during natural infection. One challenge will be to develop vaccine formulations of NspA that are more immunogenic in humans than natural infection. There are clear precedents for achieving this goal, for example tetanus toxoid or *H. influenzae* type b polysaccharide-protein conjugate vaccines. But specific new approaches may be needed for enhancing the immunogenicity of these membrane proteins.

3. *N. meningitidis* group B polysaccharide-based vaccines

The group B polysaccharide capsule is composed of a homolinear polymer of $\alpha(2\rightarrow 8)$*N*-acetyl neuraminic acid (polysialic acid). This capsule is an important virulence determinant. Mutants deficient in capsular expression are serum-sensitive and nonpathogenic [60]. Evidence also indicates that serum antibody to the group B polysaccharide confers protection against disease by activating complement-mediated bacteriolysis and/or opsonization [61]. Efforts, however, to employ the meningococcal B capsular polysaccharide as a vaccine component have been hampered by its poor immunogenicity, even when conjugated to a carrier protein [62,63]. This poor immunogenicity is attributed to immunologic tolerance induced by fetal exposure to cross-reactive polysialated glycoproteins that are expressed in a variety of host tissues, such as the neural cell adhesion molecule [64,65].

An innovative strategy for overcoming immunologic tolerance was first proposed by Jennings and coworkers. These investigators substituted *N*-propionyl (N-Pr) for *N*-acetyl (N-Ac) groups on the polysaccharide and conjugated the resulting N-Pr meningococcal B polysaccharide to a protein carrier [23,66]. The resulting conjugate vaccine was highly immunogenic in experimental animals, eliciting IgG anti-capsular antibodies that activated complement-mediated bacteriolysis in vitro and passively protected experimental animals infected with meningococcal B bacteria. A second generation version of this vaccine that uses a recombinant OMP as a carrier is currently in late-state pre-clinical development [22]. However, one important safety concern with this vaccine is that a subset of the antibodies elicited has anti-host antibody activity [22,67–69]. Autoantibodies elicited by N-Pr meningococcal B polysaccharide have the potential to either evoke autoimmune disease in vaccinated individuals or to cross the placenta and interfere with neural cell migration in the developing fetus [70,71]. Therefore, it will be a difficult task to establish that such a vaccine is safe.

The chemical structures of meningococcal B polysaccharide and host polysialic acid appear to be identical to each other [64,72]. However, based on binding data from Mabs prepared to N-Pr meningococcal B polysaccharide, there appear to exist polysaccharide-associated epitopes on the bacteria that are distinct from those present on the host polysialic acid [67,69].

We have prepared a panel of murine Mabs to N-Pr meningococcal B polysaccharide [67]. Fig. 1 shows data from indirect fluorescence flow cytometry measuring binding of murine Mabs to the surface of either live meningococcal bacteria (A) or to a neuroblastoma cell line, CHP-134, which expresses on its surface long-chain polysialic acid associated with the neuronal cell adhesion molecule (B). The latter serves as a representative cellular marker for human polysialic acid antigens. As shown in Fig. 1A, a representative anti-N-Pr meningococcal B polysaccharide Mab, designated SEAM 3, binds to an encapsulated meningococcal B strain NMB, but not to strain M7, a transposon-containing capsular-deficient mutant of the strain NMB. This result demonstrates the specificity of the Mab for capsular polysaccharide epitopes. However, as shown in Fig. 1B, the SEAM 3 anti-N-Pr meningococcal B polysaccharide Mab gives no detectable binding to the CHP-134 cells

Table 1
Peptide and antibody mimetics of carbohydrates

Carbohydrate	Mimetic	Comment	Reference(s)
α-D-Mannopyranoside	Peptide	Similar consensus sequence obtained independently by two groups	Scott et al. [100]; Oldenberg et al. [98]
Meningococcal group C polysaccharide	Anti-idiotype Mab	Elicits protective antibody when used as an immunogen	Westerink et al. [84,93]
Meningococcal group C polysaccharide	Peptide	Elicits protective antibody when used as an immunogen; peptide derived from anti-Id Mab	Westerink et al. [85]
Lewis Y antigen	Peptide	Peptide is a specific inhibitor of selecting Mab binding to cellular antigens	Hoess et al. [97]
Siayl Lewis X antigen	Peptide	Peptide is a specific inhibitor of neutrophil adhesion	Martens et al. [119]
Group A streptococcal polysaccharide	Peptides	Mab binding to peptide mimetics different than binding to polysaccharide	Harris et al. [96]
Type III group B streptococcal polysaccharide	ScFv antibody	Elicits protective antibody when used as an immunogen	Magliani et al. [79]
Type III group B streptococcal polysaccharide	Peptide	Elicits polysaccharide cross-reactive antibody when used as an immunogen	Pincus et al. [81]
Cryptococcal glucuronoxylomannan	Peptides	Peptides are good antigenic mimetics but poor immunogenic mimetics	Valadon et al. [83]; Zhang et al. [86]
P. aeruginosa lipooligosaccharide	Anti-idiotype Mab	Elicits protective antibody when used as an immunogen	Schreiber et al. [91]
N. meningitidis lipooligosaccharide	Peptide	Preliminary evidence of immunogenic mimicry	Charalambous and Feavers [120]
N. gonorrhoeae lipooligosaccharide	Anti-idiotype Mab	Elicits anti-lipooligosaccharide cross-reactive antibodies	Gulati et al. [87]
S. flexneri serotype 5 lipopolysaccharide	Peptide	Two of 19 peptides identified elicit anti-carbohydrate antibodies	Phalipon et al. [80]
Adenocarcinoma-associated carbohydrate antigens (Lewis Y)	Peptides	Evidence for immunogenic mimicry is weak (i.e. specificity not demonstrated)	Kieber-Emmons et al. [121]
HIV carbohydrate antigens	Peptides	General peptide motifs elicit carbohydrate cross-reactive antibodies	Agadjanyan et al. [107]

when tested at an antibody concentration of 100 μg ml^{-1} (approximately 98% of the cells have < 10 units of fluorescence). In contrast, a second anti-N-Pr meningococcal B polysaccharide Mab, SEAM 35, shows strong binding to the CHP-134 cells when tested at an antibody concentration of 10 μg ml^{-1}. Evidence of specificity of binding of SEAM 35 to polysialic acid is demonstrated by a decrease in fluorescence to negative control levels when this Mab is incubated with CHP-134 cells that were pre-treated with neuraminidase. Thus, there are capsular epitopes defined by SEAM 3 that are unique to the bacteria.

SEAM 3 also elicits complement-mediated bacteriolysis [67], is opsonic in vitro [73] and passively confers protection against bacteremia and meningitis in infant rats challenged with meningococcal B bacteria [73]. Hence, a vaccine that elicited serum antibodies to the antigenic specificity defined by SEAM 3 should both confer protection against meningococcal B disease and avoid the safety concerns of eliciting autoantibodies to host polysialic acid. As described below, one approach to identifying such a candidate antigen is the use of molecular mimetics

4. Molecular mimetics of polysaccharide epitopes as vaccine candidates

Recently, there has been considerable interest in using molecular mimetic immunogens to elicit protective immune responses to different pathogens [74–86]. This approach has the greatest utility when the nominal antigen is toxic or difficult to purify (i.e. the

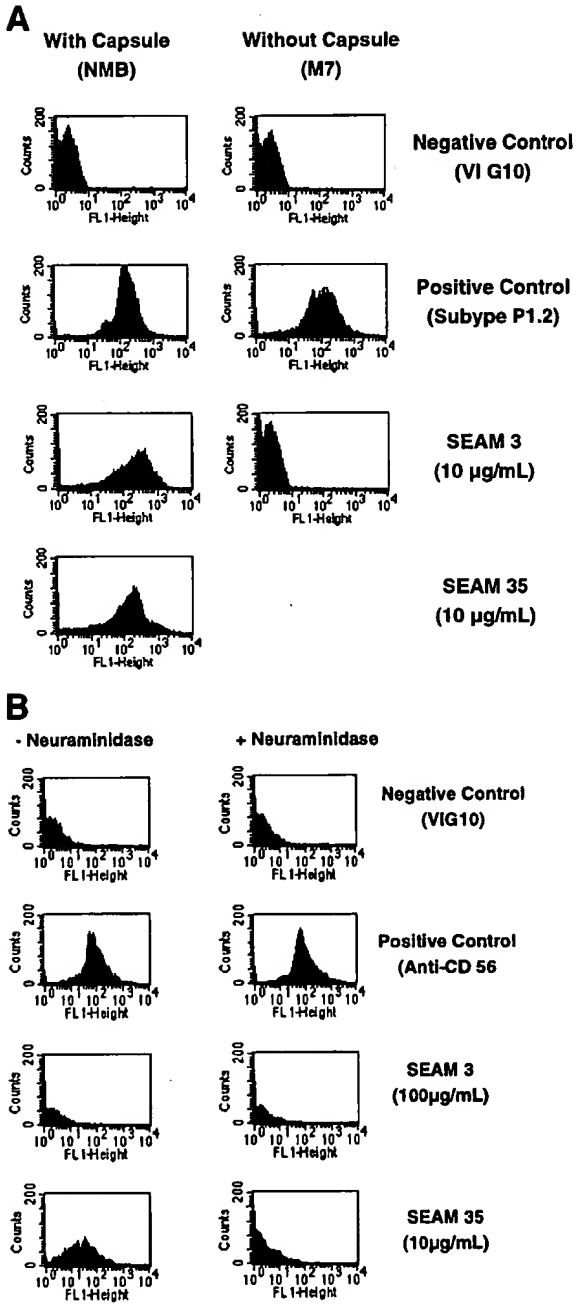

Fig. 1. Cross-reactivity of representative anti-N-Pr meningococcal B polysaccharide Mabs with encapsulated and non-encapsulated whole meningococcal B bacteria (A) and polysialic acid antigens displayed on the surface of the human neuroblastoma cell line CHP-134 (B) as determined by indirect fluorescence flow cytometry. A: Binding of VIG10, an irrelevant Mab, *N. meningitidis* subtype reagent P1.2 (Rijksinstituut Voor Volksgezondheid en Milieu, Bilthoven, The Netherlands) and an anticapsular SEAM 3 and 35 to meningococcal strains NMB and M7. The capsule of strain NMB contains N-Ac meningococcal B polysaccharide. M7 is a non-encapsulated mutant derived from strain NMB by transposon insertion. SEAM 35 was not tested with strain M7. B: Binding of VIG10 (negative control Mab, irrelevant specificity), anti-CD 56 (positive control, see text) and anti-N-Pr meningococcal B polysaccharide Mabs SEAM 3 and SEAM 35 to CHP-134 neuroblastoma cells.

lipooligosaccharide of *Neisseria gonorrhoeae* [87]) or when it is desirable to direct the immune response to a limited number of epitopes (see for example [74,82]). Table 1 summarizes reports from the literature of peptide and anti-idiotype antibody mimetics of carbohydrate structures.

4.1. Anti-idiotype internal image mimetics

Idiotypes are antigenic determinants in or near the paratope, the portion of the immunoglobulin molecule defining antibody specificity (reviewed in Bona [88] and Greenspan [89]). The antibody response to the nominal antigen is referred to as Ab1 (Fig. 2). Idiotypes on the Ab1 molecules can be effective immunogens and elicit anti-idiotype antibody responses (so called, Ab2). As shown in Fig. 2, a subset of the Ab2 responses includes antibodies (Ab2β) that express an 'internal image' of the Ab1 molecule. This

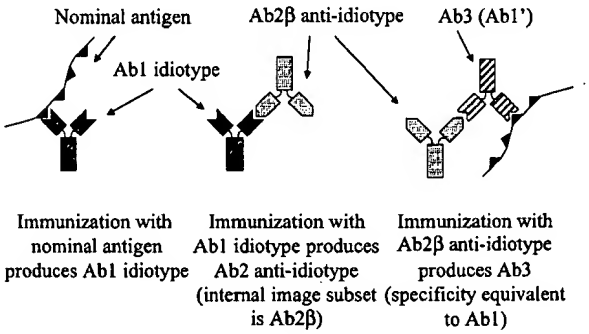

Fig. 2. Schematic depiction of the relationship between idiotype antibodies and anti-idiotype internal image antibody mimetics. The nominal antigen elicits antibody Ab1. Immunization with Ab1 elicits anti-idiotype antibodies Ab2. A subset of Ab2 antibodies known as Ab2β have surface characteristics that are internal images of the Ab1 paratope. Ab2β antibodies are defined by the ability to mimic the nominal antigen in binding to Ab1 and in eliciting antibodies (Ab3) in which a subset cross-reacts with the nominal antigen (Ab1').

'internal image' on the Ab2β molecule may effectively mimic the nominal antigen used to elicit the original Ab1 response [90] and elicit an antibody response (Ab3) cross-reactive with the nominal antigen.

Internal images expressed by anti-idiotype Ab2 antibodies can mimic carbohydrate epitopes of certain bacterial pathogens and can elicit antibody responses that are reactive with the nominal antigen expressed by the pathogen. For example, idiotype-based vaccines have been reported to elicit protective (Ab3) anti-lipooligosaccharide antibody responses to *Pseudomonas aeruginosa* [91] or *N. gonorrhoeae* [87]. Internal image idiotypes have also been reported to mimic epitopes and to elicit protective antibody to the polysaccharide capsule of *N. meningitidis* group C [92,93].

Recent advances using recombinant single chain fragment variable (ScFv) antibodies have greatly simplified the process of preparing anti-idiotype antibodies [94,95]. Furthermore, Magliani et al. recently showed that a single chain fragment Ab2 anti-idiotype, prepared against a protective anti-polysaccharide Mab, elicited a protective serum anti-carbohydrate antibody response in mice to type III, group B streptococcus [79].

4.2. Peptide mimetics of polysaccharides

Random peptide libraries expressed by filamentous phage provide another approach to identify candidate mimetic molecules [81,83,96–103]. The utility of peptide libraries for defining the fine antigenic specificity of protective and non-protective murine Mabs to a polysaccharide has been demonstrated by Valadon et al. for the *Cryptococcus neoformans* polysaccharide capsule [83]. Antigenic mimicry between peptides and cryptococcal polysaccharide epitopes has also been demonstrated by competitive binding to human antibodies [86].

The underlying principle that some antigenic mimetic peptides (i.e. binding mimicry) can also serve as immunogenic mimetics capable of eliciting anti-capsular antibodies is supported by studies of Westerink et al. [85] and Pincus et al. [81]. Westerink described a small peptide that in mice elicited complement-mediated bactericidal anti-capsular antibody responses and conferred protection against a lethal challenge by serogroup C meningococci. In addition, immunization of 'hu-PBMC-SCID' mice with the peptide mimetic elicited human anti-capsular antibody responses that were functional in a bactericidal assay [104]. This peptide was identified from the amino acid sequence corresponding to a loop segment of an anti-idiotypic antibody directed against an anti-meningococcal C polysaccharide Mab. Pincus et al. described a 12-amino acid peptide mimetic of type III group B streptococcal carbohydrate that elicited protective anti-carbohydrate antibodies in mice [81]. The peptide was selected from a phage display peptide library using a protective anti-type III group B streptococcal carbohydrate Mab.

4.3. Peptide mimetics of N-Pr meningococcal B polysaccharide epitopes

To search for peptide mimetics of meningococcal B polysaccharide, we screened phage display peptide libraries with several anti-N-Pr meningococcal B polysaccharide antibodies (SEAM Mabs). The antibodies used were heterogeneous with respect to fine antigenic specificity but were all able to activate complement-mediated bacteriolysis. They were also chosen based on no detectable cross-reactivity or minimal cross-reactivity with host polysialic acid. The phage display peptide libraries were constructed

Table 2
Characteristics of phage display peptide libraries[a]

Library	Randomized segment[b]	Number of sequences
Linear eight-mer (L8)	AEXXXXXXXXGG$(P)_6$...	2.5×10^8
Cyclic six-mer (C6)	AECXXXXXXC$(P)_4$...	6.4×10^7
Single C (C1)	AEXXXXXXXXGC$(P)_6$...	2.5×10^8

[a]Peptides are displayed as fusions with M13 phage protein, pIII.
[b]X represents a random amino acid, all others are standard single letter code.

in an M13 vector (gift of J. Winter of Chiron Corporation, Emeryville, CA, USA). As shown in Table 2, the peptides are displayed as amino-terminal extensions of the bacteriophage protein, pIII, with an intervening polyproline segment to extend the display peptide away from the protein. The linear eight-mer library consists of eight randomized codons. Since structural constraints can improve the affinity of peptide ligands in binding to antibody receptors, the cyclic six-mer and single C libraries contain two and one cysteine(s), respectively, in order to favor the selection of peptides having the potential to be constrained by a disulfide bond. All three of the libraries begin at the amino-terminus with Ala-Glu from the wild-type pIII sequence in order to ensure proper processing of the signal sequence.

4.4. Screening phage display peptide libraries with anti-N-Pr meningococcal B polysaccharide Mabs

The biopanning procedure used to screen the libraries was based on methods described by Smith and Scott [102]. In brief, Mabs were absorbed directly onto microtiter plates. After blocking the plates with 5% non-fat dry milk in phosphate-buffered saline (PBS-milk), the phage libraries were added ($\sim 10^{10}$ pfu) in PBS-milk to the plates and incubated overnight at 4°C. The plates were washed nine times with PBS and the bound phage was released by adding 0.2 M glycine-HCl (pH 2.2) buffer. The solution containing released phage was neutralized with Tris buffer (1.5 M, pH 8.8) and used to infect a fresh culture of *E. coli*. After removing the bacteria, the phage produced in the culture were harvested by precipitation with polyethylene glycol and were used in the next round of panning. The biopanning procedure was repeated four times for each Mab. Phage from the final round of panning were isolated as plaques and propagated in *E. coli*. DNA prepared from a portion of the isolated phage was used for sequencing.

To date, anti-N-Pr meningococcal B polysaccharide Mabs SEAM 2, 3, 7, 16 and 18 (Mabs described in detail in [67]) were used to screen the linear eight-mer, cyclic six-mer and the single C phage display peptide libraries. A total of 67 unique peptide sequences was selected by the anti-polysaccharide Mabs. Of these, six were identified on more than one occasion and their respective synthetic peptides were shown to be reactive with the Mabs (Tables 3 and 4). Five of the six contained two cysteines. Of these, Pep3 and 4 were identified from the cyclic six-mer library and Pep6, 8 and 9 from the single C library. As specificity controls, three additional Mabs were used to screen the single C library: a mouse IgM anti-meningococcal B polysaccharide Mab with strong autoantibody activity to human polysialic acid (2-1B [105]), a human IgM anti-*H. influenzae* type b polysaccharide Mab (ED8 [106]) and a mouse IgG Mab of irrelevant specificity (LaZ2 [105]). None of the sequences selected by the three control antibodies from the single C library was identical or significantly homologous to those selected by the anti-N-Pr meningococcal B polysaccharide Mabs (data not shown).

Although the particular sequences selected by the anti-N-Pr meningococcal B polysaccharide Mabs were variable, most of the 67 unique sequences contained a three-amino acid segment corresponding to one of two related consensus sequence motifs: hydrophobic-Arg-hydrophobic or hydrophobic-(Arg/Gln)-(Ser/Tyr/Gly). The motif occurs once or twice in the sequence. The length of the motif (three to ~six amino acids) and the prevalence of Arg in the sequence are similar to those of mimetic peptides identified for other polysaccharide binding proteins [85,96]. Arginine may mimic hydrogen bonding properties of multiple hydroxyl groups in a carbohydrate while the hydrophobic moieties may simulate interactions with the carbon backbone.

4.5. Antigenic mimicry of candidate N-Pr meningococcal B polysaccharide peptide mimetics

For characterization of the binding characteristics of the different Mabs to synthetic peptides, the partially purified anti-N-Pr meningococcal B polysaccharide Mabs were purified further on a BIOCAD perfusion chromatography workstation using a Poros G/M column. An ELISA was used to determine the ability of the Mabs to recognize synthetic peptides corresponding to selected peptide mimetic sequences. Synthetic peptides were purchased from Biosynthesis. To facilitate absorption to wells of the ELISA plate, the peptides were modified by the

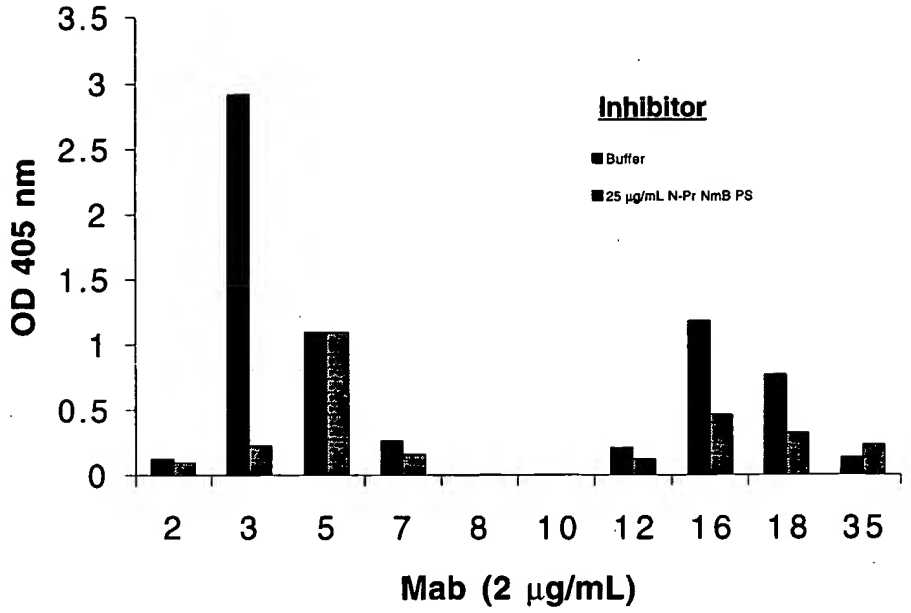

Fig. 3. Binding of anti-N-Pr meningococcal B polysaccharide Mabs to solid-phase lauroyl-Gly-Gly-Pep8 (see Table 3 for the amino acid sequence of Pep8). Data are shown for antibodies diluted in buffer or diluted in buffer plus 25 µg ml^{-1} N-Pr meningococcal B polysaccharide. The Mabs were tested at a concentration of 2 µg ml^{-1}. The results are after 30 min incubation with substrate.

addition of a hydrophobic tail moiety (lauroyl-Gly-Gly) to the amino-terminus. Further, the peptides were synthesized as carboxyl-terminal amides. Some of the synthetic lauroyl-Gly-Gly peptides showed poor binding to one or more of the corresponding Mabs used to select the respective phage display peptide (compare Tables 3 and 4). This poor binding may be due to low affinity binding or to sensitivity of the Mab/peptide binding interactions to how the peptide is presented. Fig. 3 shows representative binding data of different Mabs to the peptide Pep8. For anti-N-Pr meningococcal B polysaccharide Mab SEAM 3, the addition of an excess N-Pr meningococcal B polysaccharide (25 µg ml^{-1}) to the antibody diluting buffer completely inhibited binding to the solid-phase peptide. For the other antibodies, there was either partial inhibition of binding (e.g. SEAM 16 and 18) or no inhibition (SEAM 5). Similar results were obtained with other peptides in Table 3.

The ability of soluble polysaccharide to inhibit binding of anti-N-Pr meningococcal B polysaccharide Mab, SEAM 3, to solid-phase Pep8 was highly

Table 3
Phage display peptides selected multiple times by anti-N-Pr meningococcal B polysaccharide Mabs

Synthetic peptide designation[a]	Sequence[b]	Selecting antibody[c]	Number of identical isolates	Cross-reacting antibody[d]
Pep1	PLRSLRSYWG	SEAM 3, 16, 18	37	SEAM 16, 18
Pep3	CMRYEATC	SEAM 7	5	SEAM 3, 5, 16, 18
Pep4	CGLPRFRC	SEAM 7, 18	2	SEAM 3, 5, 16, 18
Pep6	QVPCSSRRGC	SEAM 12	4	SEAM 18
Pep8	PHCKVNRGC	SEAM 28	2	SEAM 3, 5, 16, 18
Pep9	SCRSKNSAGC	SEAM 3	2	SEAM 3

[a]Pep2, 5 and 7 are excluded because none of the selecting Mabs exhibited binding to the synthetic peptide.
[b]Underlined sequences correspond to consensus motifs of hydrophobic-Arg-hydrophobic or hydrophobic-(Arg/Gln)-(Ser/Tyr/Gly).
[c]Where Mab absorbed to microtiter plate wells is used to capture phage displaying the peptide indicated.
[d]Defined as Mab binding to the solid-phase antigen, lauroyl-Gly-Gly-Pep, in an ELISA. The Mab was tested at 2 µg ml^{-1}. A positive is defined as an OD at 405 nm of >0.5 after 30 min incubation with substrate.

specific. As little as 0.05 μg ml^{-1} N-Pr meningococcal B polysaccharide completely inhibited the binding. In contrast, 1000-fold higher concentrations of the closely related *N. meningitidis* group C polysaccharide ($\alpha(2\rightarrow 9)$*N*-acetyl neuraminic acid) did not inhibit binding (data not shown).

4.6. *Apparent relative avidity of the different antibodies for binding to the polysaccharide and to the peptide mimetics*

Dose-response binding of anti-N-Pr meningococcal B polysaccharide Mabs to solid-phase N-Pr meningococcal B polysaccharide, or to the candidate lauroyl-Gly-Gly mimetic peptides adsorbed to microtiter wells, was measured in an ELISA. Table 4 summarizes the respective concentrations of the different Mabs required to give an absorbance of 0.5 after 30 min of incubation with substrate. The respective concentrations required for binding to the polysaccharide and to the peptides were similar. See, for example, the relative binding of antibodies SEAM 3, 5, 7, 16 and 18 to the polysaccharide and to Pep8.

These results differ from those described by Harris et al. [96]. Their group employed a panel of anti-group A streptococcus cell-wall polysaccharide Mabs to select peptide mimetics from several different phage display peptide libraries. As with our collection anti-N-Pr meningococcal B polysaccharide Mabs, their collection of anti-streptococcal carbohydrate Mabs showed differences in fine antigenic specificity. In addition, their Mabs were shown to recognize a 'core' polysaccharide epitope in common. However, the peptides selected by their panel of Mabs did not have any amino acid sequence motifs in common. Further, the peptides selected were highly specific for binding only to the Mabs used to select them. These results indicate that the peptides did not mimic the 'core' polysaccharide epitope recognized in common by the panel. In contrast, the peptides selected by our Mabs cross-reacted with different Mabs, irrespective of the Mab used for selection. This extensive cross-reactivity suggests that the peptides identified by our Mabs are antigenic mimetics of a polysaccharide epitope that is recognized in common by the Mabs.

4.7. *Peptide competition of anti-capsular antibody binding*

The studies described above demonstrate that in an ELISA, soluble polysaccharide antigen can easily inhibit binding of anti-capsular Mab SEAM 3 to the candidate peptide mimetic absorbed to a solid-phase. To date, however, attempts to demonstrate the reciprocal inhibition (i.e. using soluble synthetic peptide mimetic to inhibit binding of the Mab to the polysaccharide antigen absorbed to wells of a microtiter plate) have been inconclusive. For several of the anti-N-Pr meningococcal B polysaccharide Mabs tested, instead of inhibition, we observed increased binding in the presence of soluble peptide. For example, in the presence of soluble Pep8, the ELISA readout for binding of anti-N-Pr meningococcal B polysaccharide Mab SEAM 3 to solid-phase N-Pr meningococcal B polysaccharide increased 3-fold compared to the absence of Pep8. This result appeared to be specific for Pep8 in that an increase in Mab binding was not observed in the presence of soluble, irrelevant, control peptides having similar

Table 4
Concentration-dependent binding of Mabs to solid-phase N-Pr meningococcal B polysaccharide, as compared to binding to mimetic peptides[a]

Mab	N-Pr meningococcal B polysaccharide	Pep1	Pep3	Pep4	Pep6	Pep8	Pep9
3	0.004	–	0.016	0.014	–	0.009	0.019
5	5	–	3	3	–	3	23
7	15	81	11	6	25	11	60
16	0.08	0.2	–	0.2	–	0.06	–
18	0.14	0.8	0.8	0.4	1	0.2	–

(–) Indicates no detectable binding in the ELISA. See Table 3 for the amino acid sequences of the peptides. All peptides contain an amino-terminal lauroyl-Gly-Gly group and are coated directly onto the microtiter plates.
[a]Concentration of Mab (μg ml^{-1}) required to give an OD of 0.5 at 405 nm after 30 min incubation with substrate in an ELISA.

amino acid compositions and net charges as Pep8. These data are consistent with a specific interaction of Pep8 and the Mab, perhaps formation of immune complexes that adhere to the wells of the microtiter plate and give an increased signal in the ELISA. A second explanation is low affinity binding of Pep8 with net partial occupancy of the paratope. With Pep8, this 'partial occupancy' is evidently not sufficient to inhibit binding of the anti-N-Pr meningococcal B polysaccharide Mabs to the polysaccharide. Instead, 'partial occupancy', if it occurs, appears to stabilize binding between the Mab and polysaccharide bound to a solid-phase.

Others have described similar difficulties in obtaining inhibition of Mab binding to solid-phase antigen by peptide mimetics [86,107]. In a few instances where such inhibition was reported, instead of soluble peptides, very high concentrations of phage particles displaying the peptide on phage pVIII [108] or pIII [81,82,86,109] proteins were used as the inhibitors. In one study, Valadon et al. were able to demonstrate inhibition of antibody binding to cryptococcal polysaccharide by soluble peptide mimetics [83]. However, to demonstrate inhibition, they used Fab' fragments instead of whole Ig molecules. Substitution of Fab' effectively lowers the functional affinity of binding to the solid-phase polysaccharide. Apparently, under these conditions, it is easier to demonstrate inhibition of binding by the peptide mimetic.

4.8. *Importance of peptide structural constraints in antigenic mimicry*

As noted above (Table 2), one of the randomized phage libraries used by us to search for peptide mimetics was a cyclic six-mer with two cysteines present and a second library had a single cysteine present. These two libraries were chosen specifically for their potential to select cyclic peptide structures that might adopt more stable confirmations and, therefore, be more effective mimetics of polysaccharide epitopes. However, one important finding in our studies was that for peptides that contain two cysteine residues, it was necessary that they are oxidized to be recognized by the Mabs (data not shown). These data underscore the importance of maintaining the cyclic, oxidized form of the peptide to preserve antigenic mimicry. Others have described similar results for peptide mimetics containing two cysteines [78].

4.9. *Immunogenicity of Pep8/OMP vesicles*

Westerink et al. reported that a peptide mimetic of meningococcal C polysaccharide elicited serum anticapsular antibody responses in mice [85]. The immunogen used in their studies was a peptide with an amino-terminal lauroyl group that was hydrophobically complexed with meningococcal OMP vesicles. OMP vesicles have been shown by others to have excellent adjuvant properties when tested with synthetic peptides [110]. Based on these studies and the results of Westerink [85], we prepared OMP vesicles from a capsular-deficient mutant meningococcal B strain M7 [60], for use as an adjuvant. The method used to prepare the vesicles was described by Zollinger and coworkers [27,110]. To date, immunogenicity studies have been performed with Pep8/OMP. Among the candidate antigenic mimetics, Pep8 was chosen for these initial experiments based on its cross-reactivity and dose-response binding with the different protective Mabs (Table 3 and Fig. 3). For preparation of the immunogen, Pep8 was synthesized to contain the amino-terminal lauroyl-Gly-Gly moiety to facilitate complexing to the vesicles. Also, the cysteines were converted to cystine by titration with potassium ferricyanide. Equal amounts (by mass) of oxidized Pep8 and OMP were combined in detergent and dialyzed against PBS buffer. Preparations containing 5 or 50-μg doses of peptide complexed with OMP were administered intraperitoneal with complete Freund's adjuvant to groups of four female, CD1 mice. Control animals were given OMP vesicles alone. Subsequent immunizations were repeated at 3-week intervals with incomplete Freund's adjuvant instead of the complete adjuvant used for the first dose, for a total of three immunizations.

As measured by ELISA, CD1 mice immunized with Pep8/OMP developed high anti-Pep8 antibody responses in serum obtained 4 weeks post-third immunization (Fig. 4). Specificity of binding was demonstrated by the ability of soluble Pep8 (acetyl-Pep8) to inhibit antibody binding to solid-phase lauroyl-Gly-Gly-Pep8, whereas a soluble irrelevant peptide 'PepR1' (acetyl-Gln-Trp-Glu-Arg-Thr-Tyr-amide) gave no significant inhibition of antibody binding.

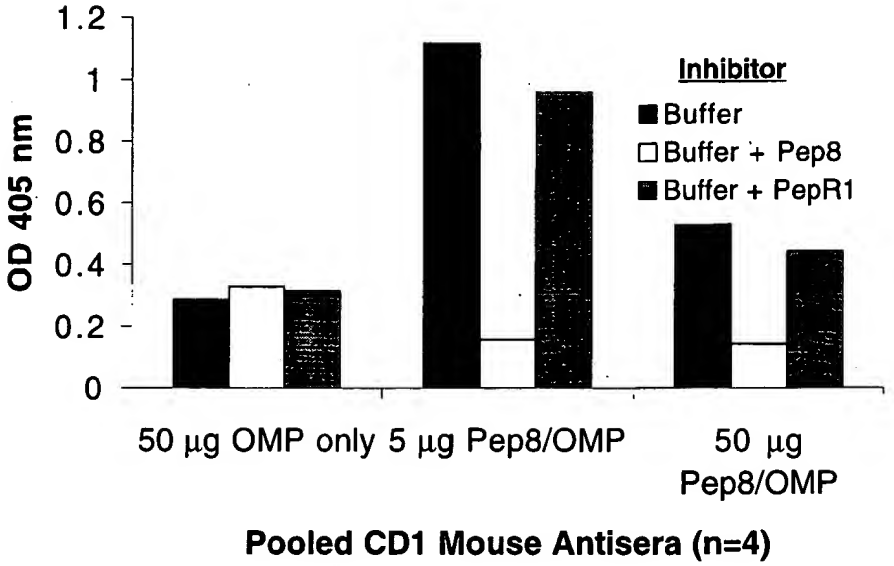

Fig. 4. Binding of anti-Pep8/OMP and anti-OMP sera to lauroyl-Gly-Gly-Pep8 as determined by ELISA. Groups of mice were immunized with different doses of Pep8 complexed to OMP vesicles or OMP vesicles alone. After three injections, respective post-immunization sera from animals in different groups were pooled. Shown are the binding data from testing dilutions of post-immune sera to Pep8/OMP (1:48 000) or control OMP without the addition of peptide (1:100). Serum pools were diluted in buffer alone, buffer containing 50 µg ml^{-1} soluble acetyl-Pep8 or buffer containing 50 µg ml^{-1} of an irrelevant peptide (PepR1, acetyl-Gln-Trp-Glu-Arg-Thr-Tyr-amide). The results are after 30 min incubation with substrate.

Specificity of the anti-Pep8 response also was shown by absence of anti-Pep8 binding in sera from control animals immunized with OMP vesicles alone. However, the antisera from mice immunized with Pep8/OMP that contained high titers of anti-Pep8 antibody (> 1:100 000) did not bind to the N-Pr meningococcal B polysaccharide nominal antigen, as measured in an ELISA (data not shown). Thus, although the anti-N-Pr meningococcal B polysaccharide Mabs recognize Pep8, the anti-Pep8 antibodies generated by immunization with lauroyl-Gly-Gly-Pep8/OMP were not comparable in polysaccharide binding to that of the anti-capsular Mab.

The lack of binding of the anti-Pep8 sera to the nominal polysaccharide antigen was not unexpected. As described above, others have had to test large numbers of antigenic mimetic peptides to identify a few that were effective immunogenic mimetics [80]. The reasons why some antigenic mimetics can serve as immunogenic mimetics while others do not are unknown. For example, Phalipon et al. [80] identified 19 peptide antigenic mimetics of *Shigella flexneri* serotype 5a lipopolysaccharide. Of these, only two were able to induce anti-O-antigen antibody responses in mice.

One possible explanation for the failure of some antigen peptide mimetics to be effective immunogen mimetics is the limited ability of small peptides to adopt stable confirmations that could mimic polysaccharide epitopes and bind to the B-cell immunoglobulin receptor with high affinity [103,111]. In a series of papers on peptide mimetics of the capsular glucuronoxylomannan (GXM) of *C. neoformans*, Scharff and coworkers have described such an example [83,111,112]. A peptide mimetic (peptide PA1) identified in a phage display peptide library by the anti-cryptococcal polysaccharide Mab 2H1 was shown to be a good antigenic mimetic in binding to 2H1 and inhibiting binding of 2H1 Fab' to polysaccharide [83]. However, PA1 and related peptides were found to be poor immunogenic mimetics in failing to elicit significant titers of GXM cross-reactive antibody [111,112]. Several possible reasons for the poor immunogenic mimicry of PA1 and related peptides were revealed by the X-ray crystallographic structure of 2H1 Fab' with and without peptide

PA1 bound to it [112]. The crystal structure clearly shows the peptide within the antibody combining site. However, the contacts between the peptide and the antibody are almost exclusively non-specific, van der Waals interactions. Furthermore, there is poor steric complementarity between PA1 and 2H1. Most importantly, it is unlikely that a significant population of the tightly coiled conformation of the peptide bound to the Mab would exist in aqueous solution. Hence, the affinity of PA1 for equivalent Ig receptors on antigen presenting cells is likely to be weak. As suggested by Scharff and coworkers, it may be especially important for peptide mimetics of carbohydrate epitopes to have a stable structure closely resembling that of the epitope in order to be an effective immunogenic mimetic [112].

With increased recognition of the importance of structural considerations in immunogenic mimicry, we are searching for peptide mimetics of N-Pr meningococcal B polysaccharide in new phage libraries that display independently folding αβ and ββ peptides [113–115]. We are also using the anti-N-Pr meningococcal B polysaccharide Mabs to screen pools of combinatorial small molecules. Although this work is at a preliminary stage, we are hopeful that some of the small molecules identified may be immunogenic mimetics of meningococcal B polysaccharide-specific epitopes when conjugated to carrier proteins. Potentially, these or related approaches will lead to the discovery of molecular mimetic antigens that can elicit protective anti-capsular antibody responses to the meningococcal B organism but avoid eliciting potentially harmful autoantibodies to host polysialic acid.

5. Application of genomic studies to antigen discovery

As described above, the goal of a safe and effective universal meningococcal B vaccine remains elusive and the scientific challenges in developing such a vaccine are formidable. New approaches, therefore, are needed. As described in the Section 1, most of the non-capsular antigens that are immunogenic during infection and present in the OM in a high copy number have already been identified by conventional microbiological approaches and investigated. Analysis of genomic sequences of *N. meningitidis* group B provides an opportunity to identify and investigate novel gene products that are predicted to be conserved membrane proteins and, that up to now, have gone undetected by conventional approaches.

Recently, the Institute for Genomic Research (TIGR) reported that they had nearly completed the sequencing of the whole genome of *N. meningitidis* group B strain MC58 [116]. Publication of the results is expected during 1999. Provisional data have been analyzed by Dr Rino Rappuoli's group at Chiron Vaccines (Siena, Italy) for evidence of surface-accessible proteins and conservation of predicted amino acid sequences, compared to those derived from the genomic sequences of strains of *N. meningitidis* group A [117] and *N. gonorrhoeae* [118]. More than 300 DNA sequences were identified that were predicted to encode surface proteins in *N. meningitidis* group B strain MC58 and that also are conserved in these other two organisms. Many of these genes were cloned into expression vectors. Mice were immunized with the purified recombinant proteins and the resulting antisera were tested for their ability to bind to the meningococcal bacterial surface using indirect fluorescence flow cytometry and their ability to mediate lysis of *N. meningitidis* group B bacteria in the presence of complement. Approximately 20 new candidate proteins have been identified by this approach. Given this large number of potential candidates, one may be cautiously optimistic that use of one or more of these membrane proteins, possibly in concert with a peptide mimetic antigen of the group B polysaccharide or detoxified lipooligosaccharide [42–46], will lead to the development of a safe and effective vaccine that will be suitable for universal immunization against meningococcal B disease.

References

[1] Halstensen, A., Pedersen, S.H., Haneberg, B., Bjorvatn, B. and Solberg, C.O. (1987) Case fatality of meningococcal disease in western Norway. Scand. J. Infect. Dis. 19, 35–42.
[2] Jones, D. (1995) Epidemiology of meningococcal disease in Europe and the USA. In: Meningococcal Disease (Cartwright, K., Ed.), pp. 147–175. John Wiley and Sons, New York.
[3] Peltola, H., Jonsdottir, K., Lystad, A., Sievers, C.J. and Kallings, I. (1982) Meningococcal disease in Scandinavia. Br. Med. J. 284, 1618–1621.

[4] Scholten, R.J., Bijlmer, H.A., Poolman, J.T., Kuipers, B., Caugant, D.A., Van Alphen, L., Dankert, J. and Valkenburg, H.A. (1993) Meningococcal disease in The Netherlands, 1958-1990: a steady increase in the incidence since 1982 partially caused by new serotypes and subtypes of *Neisseria meningitidis*. Clin. Infect. Dis. 16, 237–246.

[5] Naess, A., Halstensen, A., Nyland, H., Pedersen, S.H., Moller, P., Borgmann, R., Larsen, J.L. and Haga, E. (1994) Sequelae one year after meningococcal disease. Acta Neurol. Scand. 89, 139–142.

[6] Davis, B.D., Dulbecco, R., Eisen, H.N. and Ginsberg, H.S. (1980) The Neisseriae. In: Microbiology, 3rd edn. (Gotschlich, E.C., Ed.), pp. 635–644. Harper, New York.

[7] Ramsay, M., Kaczmarski, E., Rush, M., Mallard, R., Farrington, P. and White, J. (1997) Changing patterns of case ascertainment and trends in meningococcal disease in England and Wales. Commun. Dis. Rep. CDR Rev. 7, 49–54.

[8] Moore, P.S. and Broome, C.V. (1994) Cerebrospinal meningitis epidemics. Sci. Am. 271, 38–45.

[9] Hart, C.A. and Cuevas, L.E. (1997) Meningococcal disease in Africa. Ann. Trop. Med. Parasitol. 91, 777–785.

[10] Serogroup B meningococcal disease–Oregon, 1994 (1995) Morb. Mortal. Wkly. Rep. 44, pp. 121–124.

[11] Jackson, L.A., Schuchat, A., Reeves, M.W. and Wenger, J.D. (1995) Serogroup C meningococcal outbreaks in the United States. An emerging threat. JAMA 273, 383–389.

[12] Whalen, C.M., Hockin, J.C., Ryan, A. and Ashton, F. (1995) The changing epidemiology of invasive meningococcal disease in Canada, 1985 through 1992. Emergence of a virulent clone of *Neisseria meningitidis*. JAMA 273, 390–394.

[13] Martin, D.R., Walker, S.J., Baker, M.G. and Lennon, D.R. (1998) New Zealand epidemic of meningococcal disease identified by a strain with phenotype B:4:P1.4. J. Infect. Dis. 177, 497–500.

[14] Sacchi, C.T., Pessoa, L.L., Ramos, S.R., Milagres, L.G., Camargo, M.C., Hidalgo, N.T., Melles, C.E., Caugant, D.A. and Frasch, C.E. (1992) Ongoing group B *Neisseria meningitidis* epidemic in Sao Paulo, Brazil, due to increased prevalence of a single clone of the ET-5 complex. J. Clin. Microbiol. 30, 1734–1738.

[15] Frasch, C.E. (1987) Prospects for the prevention of meningococcal disease: special reference to group B. Vaccine 5, 3–4.

[16] Jafari, H.S., Perkins, B.A. and Wenger, J.D. (1997) Control and prevention of meningococcal disease: recommendations of the Advisory Committee on Immunization Practices. Morb. Mortal. Wkly. Rep. 46, 1–10.

[17] Peltola, H. (1998) Meningococcal vaccines. Current status and future possibilities. Drugs 55, 347–366.

[18] Fairley, C.K., Begg, N., Borrow, R., Fox, A.J., Jones, D.M. and Cartwright, K. (1996) Conjugate meningococcal serogroup A and C vaccine: reactogenicity and immunogenicity in United Kingdom infants. J. Infect. Dis. 174, 1360–1363.

[19] Leach, A., Twumasi, P.A., Kumah, S., Banya, W.S., Jaffar, S., Forrest, B.D., Granoff, D.M., LiButti, D.E., Carlone, G.M., Pais, L.B., Broome, C.V. and Greenwood, B.M. (1997) Induction of immunologic memory in Gambian children by vaccination in infancy with a group A plus group C meningococcal polysaccharide-protein conjugate vaccine. J. Infect. Dis. 175, 200–204.

[20] MacDonald, N.E., Halperin, S.A., Law, B.J., Forrest, B., Danzig, L.E. and Granoff, D.M. (1998) Induction of immunologic memory by conjugated vs plain meningococcal C polysaccharide vaccine in toddlers: a randomized controlled trial. JAMA 280, 1685–1689.

[21] Campagne, G., Garba, A., Fabre, P., Carlone, G., Ryall, B., Froeschle, J., Schuchat, A., Boulanger, D., Briantais, P. and Chippaux, J.P. (1997) Safety and immunogenicity of three doses of a *N. meningitidis* A/C diphtheria conjugate vaccine in infants in Niger. In: 37th Interscience Conference on Antimicrobial Agents and Chemotherapy, p. 192. American Society for Microbiology, Toronto.

[22] Fusco, P.C., Michon, F., Tai, J.Y. and Blake, M.S. (1997) Preclinical evaluation of a novel group B meningococcal conjugate vaccine that elicits bactericidal activity in both mice and nonhuman primates. J. Infect. Dis. 175, 364–372.

[23] Jennings, H.J., Gamian, A. and Ashton, F.E. (1987) N-propionylated group B meningococcal polysaccharide mimics a unique epitope on group B *Neisseria meningitidis*. J. Exp. Med. 165, 1207–1211.

[24] Bjune, G., Gronnesby, J.K., Hoiby, E.A., Closs, O. and Nokleby, H. (1991) Results of an efficacy trial with an outer membrane vesicle vaccine against systemic serogroup B meningococcal disease in Norway. NIPH Ann. 14, 125–130.

[25] Boslego, J., Garcia, J., Cruz, C., Zollinger, W., Brandt, B., Ruiz, S., Martinez, M., Arthur, J., Underwood, P. and Silva, W. et al. (1995) Efficacy, safety, and immunogenicity of a meningococcal group B (15:P1.3) outer membrane protein vaccine in Iquique, Chile. Chilean National Committee for Meningococcal Disease. Vaccine 13, 821–829.

[26] Perkins, B.A., Jonsdottir, K., Briem, H., Griffiths, E., Plikaytis, B.D., Hoiby, E.A., Rosenqvist, E., Holst, J., Nokleby, H., Sotolongo, F., Sierra, G., Campa, H.C., Carlone, G.M., Williams, D., Dykes, J., Kapczynski, D., Tikhomirov, E., Wenger, J.D. and Broome, C.V. (1998) Immunogenicity of two efficacious outer membrane protein-based serogroup B meningococcal vaccines among young adults in Iceland. J. Infect. Dis. 177, 683–691.

[27] Zollinger, W.D., Boslego, J., Froholm, L.O., Ray, J.S., Moran, E.E. and Brandt, B.L. (1987) Human bactericidal antibody response to meningococcal outer membrane protein vaccines. Antonie Van Leeuwenhoek 53, 403–411.

[28] Borrow, R., Clark, S., Sadler, F., Fox, A.J., van der Ley, P. and van der Voort, E.R. (1998) Effect of sequence variation in the meningococcal PorA OMP on the immungenicity of a recombinant PorA meningococcal OMV vaccine in UK infants. In: Abstracts of the Eleventh International Pathogenic Neisseria Conference (Nassif, X., Quentin-Millet, M.-J. and Taha, M.-K., Eds.), p. 178. Editions E.D.K., Paris, France.

[29] Christodoulides, M., Brooks, J.L., Rattue, E. and Heckels, J.E. (1998) Immunization with recombinant class 1 outer-membrane protein from *Neisseria meningitidis*: influence of liposomes and adjuvants on antibody avidity, recognition of native protein and the induction of a bactericidal immune response against meningococci. Microbiology 144, 3027–3037.

[30] Idänpään-Heikkilä, I., Muttilainen, S., Wahlström, E., Saarinen, L., Leinonen, M., Sarvas, M. and Mäkelä, P.H. (1995) The antibody response to a prototype liposome vaccine containing *Neisseria meningitidis* outer membrane protein P1 produced in *Bacillus subtilis*. Vaccine 13, 1501–1508.

[31] Muttilainen, S., Idänpään-Heikkilä, I., Wahlström, E., Nurminen, M., Mäkelä, P.H. and Sarvas, M. (1995) The *Neisseria meningitidis* outer membrane protein P1 produced in *Bacillus subtilis* and reconstituted into phospholipid vesicles elicits antibodies to native P1 epitopes. Microb. Pathog. 18, 423–436.

[32] Peeters, C.C., Rumke, H.C., Sundermann, L.C., van der Voort, E.M.R., Meulenbelt, J., Schuller, M., Kuipers, A.J., van der Ley, P. and Poolman, J.T. (1996) Phase I clinical trial with a hexavalent PorA containing meningococcal outer membrane vesicle vaccine. Vaccine 14, 1009–1015.

[33] van der Voort, E.M.R., Kuipers, B., Brugghe, H.F., van Unen, L.M., Timmermans, H.A., Hoogerhout, P. and Poolman, J.T. (1997) Epitope specificity of murine and human bactericidal antibodies against PorA P1.7, 16 induced with experimental meningococcal group B vaccines. FEMS Immunol. Med. Microbiol. 17, 139–148.

[34] van der Voort, E.R., van der Ley, P., van der Biezen, J., George, S., Tunnela, O., van Dijken, H., Kuipers, B. and Poolman, J. (1996) Specificity of human bactericidal antibodies against PorA P1.7, 16 induced with a hexavalent meningococcal outer membrane vesicle vaccine. Infect. Immun. 64, 2745–2751.

[35] Banerjee-Bhatnagar, N. and Frasch, C.E. (1990) Expression of *Neisseria meningitidis* iron-regulated outer membrane proteins, including a 70-kilodalton transferrin receptor, and their potential for use as vaccines. Infect. Immun. 58, 2875–2881.

[36] Lissolo, L., Maitre-Wilmotte, G., Dumas, P., Mignon, M., Danve, B. and Quentin-Millet, M.J. (1995) Evaluation of transferrin-binding protein 2 within the transferrin-binding protein complex as a potential antigen for future meningococcal vaccines. Infect. Immun. 63, 884–890.

[37] Pettersson, A., Kuipers, B., Pelzer, M., Verhagen, E., Tiesjema, R.H., Tommassen, J. and Poolman, J.T. (1990) Monoclonal antibodies against the 70-kilodalton iron-regulated protein of *Neisseria meningitidis* are bactericidal and strain specific. Infect. Immun. 58, 3036–3041.

[38] Rosenqvist, E., Musacchio, A., Aase, A., Hoiby, E.A., Namork, E., Kolberg, J., Wedege, E., Delvig, A., Dalseg, R., Michaelsen, T.E. and Tommassen, J. (1999) Functional activities and epitope specificity of human and murine antibodies against the class 4 outer membrane protein (Rmp) of *Neisseria meningitidis*. Infect. Immun. 67, 1267–1276.

[39] Musacchio, A., Carmenate, T., Delgado, M. and Gonzalez, S. (1997) Recombinant Opc meningococcal protein, folded in vitro, elicits bactericidal antibodies after immunization. Vaccine 15, 751–758.

[40] Martin, D., Cadieux, N., Hamel, J. and Brodeur, B.R. (1997) Highly conserved *Neisseria meningitidis* surface protein confers protection against experimental infection. J. Exp. Med. 185, 1173–1183.

[41] Martin, D., Roiux, C.R., Villeneuve, A., Hamel, J. and Brodeur, B.R. (1998) Induction of cross-reactive bactericidal and protective antibodies in animals immunized with purified recombinant meningococcal NspA protein. In: Abstracts of the Eleventh International Pathogenic Neisseria Conference (Nassif, X., Quentin-Millet, M.-J. and Taha, M.-K., Eds.), p. 198. Editions E.D.K., Paris, France.

[42] Jennings, H.J., Lugowski, C. and Ashton, F.E. (1984) Conjugation of meningococcal lipopolysaccharide R-type oligosaccharides to tetanus toxoid as route to a potential vaccine against group B *Neisseria meningitidis*. Infect. Immun. 43, 407–412.

[43] Jennings, H.J., Beurret, M., Gamian, A. and Michon, F. (1987) Structure and immunochemistry of meningococcal lipopolysaccharides. Antonie Van Leeuwenhoek 53, 519–522.

[44] Gu, X.X. and Tsai, C.M. (1993) Preparation, characterization, and immunogenicity of meningococcal lipooligosaccharide-derived oligosaccharide-protein conjugates. Infect. Immun. 61, 1873–1880.

[45] Moxon, E.R., Hood, D. and Richards, J. (1998) Bacterial lipopolysaccharides: candidate vaccines to prevent *Neisseria meningitidis* and *Haemophilus influenzae* infections. Adv. Exp. Med. Biol. 435, 237–243.

[46] Verheul, A.F., Snippe, H. and Poolman, J.T. (1993) Meningococcal lipopolysaccharides: virulence factor and potential vaccine component. Microbiol. Rev. 57, 34–49.

[47] Frasch, C.E. (1995) Meningococcal vaccines: past, present and future. In: Meningococcal Disease (Cartwright, K., Ed.), pp. 245–283. John Wiley and Sons, New York.

[48] Bjune, G., Hoiby, E.A., Gronnesby, J.K., Arnesen, O., Fredriksen, J.H., Halstensen, A., Holten, E., Lindbak, A.K., Nokleby, H. and Rosenqvist, E. et al. (1991) Effect of outer membrane vesicle vaccine against group B meningococcal disease in Norway. Lancet 338, 1093–1096.

[49] de Moraes, J.C., Perkins, B.A., Camargo, M.C., Hidalgo, N.T., Barbosa, H.A., Sacchi, C.T., Landgraf, I.M., Gattas, V.L., Vasconcelos, H.d.G. and Gral, I.M. et al. (1992) Protective efficacy of a serogroup B meningococcal vaccine in Sao Paulo, Brazil. Lancet 340, 1074–1078.

[50] Tappero, J.W. et al. (1999) Immunogenicity of 2 serogroup B outer-membrane protein meningococcal vaccines: a randomized controlled trial in Chile. JAMA 281, 1520–1527.

[51] van der Ley, P., Heckels, J.E., Virji, M., Hoogerhout, P. and Poolman, J.T. (1991) Topology of outer membrane porins in pathogenic *Neisseria* spp.. Infect. Immun. 59, 2963–2971.

[52] Poolman, J.T., Van Der Ley, P.A. and Tommassen, J. (1995) Surface structures and secreted products of Meningococci. In: Meningococcal Disease (Cartwright, K., Ed.), pp. 21–34. John Wiley and Sons, New York.

[53] Rümke, H.C., Labadie, J., van den Dobbelsteen, G. and van Alphen, L. (1998) Clinical studies with RIVM hexavalent meningococcal PorA vesicle vaccine. In: Abstracts of the Eleventh International Pathogenic Neisseria Conference (Nassif, X., Quentin-Millet, M.-J. and Taha, M.-K., Eds.), p. 58. Editions E.D.K., Paris, France.

[54] Anderson, R.M., Donnelly, C.A. and Gupta, S. (1997) Vaccine design, evaluation, and community-based use for antigenically variable infectious agents. Lancet 350, 1466–1470.

[55] Rokbi, B., Mignon, M., Caugant, D.A. and Quentin-Millet,

M.J. (1997) Heterogeneity of tbpB, the transferrin-binding protein B gene, among serogroup B *Neisseria meningitidis* strains of the ET-5 complex. Clin. Diagn. Lab. Immunol. 4, 522–529.

[56] Ala'Aldeen, D.A. (1996) Transferrin receptors of *Neisseria meningitidis*: promising candidates for a broadly cross-protective vaccine. J. Med. Microbiol. 44, 237–243.

[57] Ala'Aldeen, D.A. and Borriello, S.P. (1996) The meningococcal transferrin-binding proteins 1 and 2 are both surface exposed and generate bactericidal antibodies capable of killing homologous and heterologous strains. Vaccine 14, 49–53.

[58] Danve, B., Lissolo, L., Guinet, F., Boutry, E., Speck, D., Cadoz, M., Nassif, X. and Quentin-Millet, M.J. (1998) Safety and immunogenicity of a *Neisseria meningitidis* group B transferrin binding protein vaccine in adults. In: Abstracts of the Eleventh International Pathogenic Neisseria Conference (Nassif, X., Quentin-Millet, M.-J. and Taha, M.-K., Eds.), p. 53. Editions E.D.K., Paris, France.

[59] Farrant, J.L., Kroll, J.S., Brodeur, B.R. and Martin, D. (1998) Detection of anti-NspA antibodies in sera from patients convalescent after meningococcal infection. In: Abstracts of the Eleventh International Pathogenic Neisseria Conference (Nassif, X., Quentin-Millet, M.-J. and Taha, M.-K., Eds.), p. 208. Editions E.D.K., Paris, France.

[60] Stephens, D.S., Swartley, J.S., Kathariou, S. and Morse, S.A. (1991) Insertion of Tn916 in *Neisseria meningitidis* resulting in loss of group B capsular polysaccharide. Infect. Immun. 59, 4097–4102.

[61] Griffiss, J.M. (1995) Mechanisms of host immunity. In: Meningococcal Disease (Cartwright, K., Ed.), pp. 35–70. John Wiley and Sons, New York.

[62] Devi, S.J., Zollinger, W.D., Snoy, P.J., Tai, J.Y., Costantini, P., Norelli, F., Rappuoli, R. and Frasch, C.E. (1997) Preclinical evaluation of group B *Neisseria meningitidis* and *Escherichia coli* K92 capsular polysaccharide-protein conjugate vaccines in juvenile rhesus monkeys. Infect. Immun. 65, 1045–1052.

[63] Jennings, H.J. and Lugowski, C. (1981) Immunochemistry of groups A, B and C meningococcal polysaccharide-tetanus toxoid conjugates. J. Immunol. 127, 1011–1018.

[64] Finne, J., Finne, U., Deagostini-Bazin, H. and Goridis, C. (1983) Occurrence of alpha 2-8 linked polysialosyl units in a neural cell adhesion molecule. Biochem. Biophys. Res. Commun. 112, 482–487.

[65] Finne, J., Bitter-Suermann, D., Goridis, C. and Finne, U. (1987) An IgG monoclonal antibody to group B meningococci cross-reacts with developmentally regulated polysialic acid units of glycoproteins in neural and extraneural tissues. J. Immunol. 138, 4402–4407.

[66] Jennings, H.J., Roy, R. and Gamian, A. (1986) Induction of meningococcal group B polysaccharide-specific IgG antibodies in mice by using an N-propionylated B polysaccharide-tetanus toxoid conjugate vaccine. J. Immunol. 137, 1708–1713.

[67] Granoff, D.M., Bartoloni, A., Ricci, S., Gallo, E., Rosa, D., Ravenscroft, N., Guarnieri, V., Seid, R.C., Shan, A., Usinger, W.R., Tan, S., McHugh, Y.E. and Moe, G.R. (1998) Bactericidal monoclonal antibodies that define unique meningococcal B polysaccharide epitopes that do not cross-react with human polysialic acid. J. Immunol. 160, 5028–5036.

[68] Hayrinen, J., Jennings, H., Raff, H.V., Rougon, G., Hanai, N., Gerardy-Schahn, R. and Finne, J. (1995) Antibodies to polysialic acid and its N-propyl derivative: binding properties and interaction with human embryonal brain glycopeptides. J. Infect. Dis. 171, 1481–1490.

[69] Pon, R.A., Lussier, M., Yang, Q.L. and Jennings, H.J. (1997) N-Propionylated group B meningococcal polysaccharide mimics a unique bactericidal capsular epitope in group B *Neisseria meningitidis*. J. Exp. Med. 185, 1929–1938.

[70] Cremer, H., Lange, R., Christoph, A., Plomann, M., Vopper, G., Roes, J., Brown, R., Baldwin, S., Kraemer, P. and Scheff, S. et al. (1994) Inactivation of the N-CAM gene in mice results in size reduction of the olfactory bulb and deficits in spatial learning. Nature 367, 455–459.

[71] Rutishauser, U. (1992) NCAM and its polysialic acid moiety: a mechanism for pull/push regulation of cell interactions during development? Dev. Suppl., pp. 99–104.

[72] Bhattacharjee, A.K., Jennings, H.J., Kenny, C.P., Martin, A. and Smith, I.C.P. (1975) Structural determination of the sialic acid polysaccharide antigens of *Neisseria meningitidis* serogroups B and C with carbon 13 nuclear magnetic resonance. J. Biol. Chem. 250, 1926–1932.

[73] Verheul, A.F.M., Käyhty, H., Benaissa-Trouw, B., Snippe, H., Saarinen, L., Moe, G.R. and Granoff, D.M. (1998) Opsonophagocytic and protective capacity of murine monoclonal antibodies to N-propionyl group B meningococcal polysaccharide (N-Pr MenB PS) of different fine specificity. In: Neisseria '98. Paris, France.

[74] Chargelegue, D., Obeid, O.E., Hsu, S.C., Shaw, M.D., Denbury, A.N., Taylor, G. and Steward, M.W. (1998) A peptide mimic of a protective epitope of respiratory syncytial virus selected from a combinatorial library induces virus-neutralizing antibodies and reduces viral load in vivo. J. Virol. 72, 2040–2046.

[75] Chen, Y.C., Delbrook, K., Dealwis, C., Mimms, L., Mushahwar, I.K. and Mandecki, W. (1996) Discontinuous epitopes of hepatitis B surface antigen derived from a filamentous phage peptide library. Proc. Natl. Acad. Sci. USA 93, 1997–2001.

[76] Demangel, C., Rouyre, S., Alzari, P.M., Nato, F., Longacre, S., Lafaye, P. and Mazie, J.C. (1998) Phage-displayed mimotopes elicit monoclonal antibodies specific for a malaria vaccine candidate. Biol. Chem. 379, 65–70.

[77] El Kasmi, K.C., Theisen, D., Brons, N.H. and Muller, C.P. (1998) The molecular basis of virus crossreactivity and neutralisation after immunisation with optimised chimeric peptides mimicking a putative helical epitope of the measles virus hemagglutinin protein. Mol. Immunol. 35, 905–918.

[78] Lundin, K., Samuelsson, A., Jansson, M., Hinkula, J., Wahren, B., Wigzell, H. and Persson, M.A. (1996) Peptides isolated from random peptide libraries on phage elicit a neutralizing anti-HIV-1 response: analysis of immunological mimicry. Immunology 89, 579–586.

[79] Magliani, W., Polonelli, L., Conti, S., Salati, A., Rocca, P.F., Cusumano, V., Mancuso, G. and Teti, G. (1998) Neonatal mouse immunity against group B streptococcal infection by

maternal vaccination with recombinant anti-idiotypes. Nat. Med. 4, 705–709.

[80] Phalipon, A., Folgori, A., Arondel, J., Sgaramella, G., Fortugno, P., Cortese, R., Sansonetti, P.J. and Felici, F. (1997) Induction of anti-carbohydrate antibodies by phage library-selected peptide mimics. Eur. J. Immunol. 27, 2620–2625.

[81] Pincus, S.H., Smith, M.J., Jennings, H.J., Burritt, J.B. and Glee, P.M. (1998) Peptides that mimic the group B streptococcal type III capsular polysaccharide antigen. J. Immunol. 160, 293–298.

[82] Scala, G., Chen, X., Liu, W., Telles, J.N., Cohen, O.J., Vaccarezza, M., Igarashi, T. and Fauci, A.S. (1999) Selection of HIV-specific immunogenic epitopes by screening random peptide libraries with HIV-1-positive sera. J. Immunol. 162, 6155–6161.

[83] Valadon, P., Nussbaum, G., Boyd, L.F., Margulies, D.H. and Scharff, M.D. (1996) Peptide libraries define the fine specificity of anti-polysaccharide antibodies to *Cryptococcus neoformans*. J. Mol. Biol. 261, 11–22.

[84] Westerink, M.A., Campagnari, A.A., Wirth, M.A. and Apicella, M.A. (1988) Development and characterization of an anti-idiotype antibody to the capsular polysaccharide of *Neisseria meningitidis* serogroup C. Infect. Immun. 56, 1120–1127.

[85] Westerink, M.A., Giardina, P.C., Apicella, M.A. and Kieber-Emmons, T. (1995) Peptide mimicry of the meningococcal group C capsular polysaccharide. Proc. Natl. Acad. Sci. USA 92, 4021–4025.

[86] Zhang, H., Zhong, Z. and Pirofski, L.A. (1997) Peptide epitopes recognized by a human anti-cryptococcal glucuronoxylomannan antibody. Infect. Immun. 65, 1158–1164.

[87] Gulati, S., McQuillen, D.P., Sharon, J. and Rice, P.A. (1996) Experimental immunization with a monoclonal anti-idiotope antibody that mimics the *Neisseria gonorrhoeae* lipooligosaccharide epitope 2C7. J. Infect. Dis. 174, 1238–1248.

[88] Bona, C.A. (1998) Idiotype vaccines: forgotten but not gone. Nat. Med. 4, 668–669.

[89] Greenspan, N.S. (1997) Reflections on internal images. Nat. Biotechnol. 15, 123–124.

[90] Nisonoff, A. and Lamoyi, E. (1981) Implications of the presence of an internal image of the antigen in anti-idiotypic antibodies: possible application to vaccine production. Clin. Immunol. Immunopathol. 21, 397–406.

[91] Schreiber, J.R., Nixon, K.L., Tosi, M.F., Pier, G.B. and Patawaran, M.B. (1991) Anti-idiotype-induced, lipopolysaccharide-specific antibody response to *Pseudomonas aeruginosa. II. Isotype and functional activity of the anti-idiotype-induced antibodies*. J. Immunol. 146, 188–193.

[92] Westerink, M.A. and Apicella, M.A. (1993) Anti-idiotypic antibodies as vaccines against carbohydrate antigens. Springer Semin. Immunopathol. 15, 227–234.

[93] Westerink, M.A. and Giardina, P.C. (1992) Anti-idiotype induced protection against *Neisseria meningitidis* serogroup C bacteremia. Microb. Pathog. 12, 19–26.

[94] Barbas, C.F.d., Kang, A.S., Lerner, R.A. and Benkovic, S.J. (1991) Assembly of combinatorial antibody libraries on phage surfaces: the gene III site. Proc. Natl. Acad. Sci. USA 88, 7978–7982.

[95] Clackson, T., Hoogenboom, H.R., Griffiths, A.D. and Winter, G. (1991) Making antibody fragments using phage display libraries. Nature 352, 624–628.

[96] Harris, S.L., Craig, L., Mehroke, J.S., Rashed, M., Zwick, M.B., Kenar, K., Toone, E.J., Greenspan, N., Auzanneau, F.I., Marino-Albernas, J.R., Pinto, B.M. and Scott, J.K. (1997) Exploring the basis of peptide-carbohydrate crossreactivity: evidence for discrimination by peptides between closely related anti-carbohydrate antibodies. Proc. Natl. Acad. Sci. USA 94, 2454–2459.

[97] Hoess, R., Brinkmann, U., Handel, T. and Pastan, I. (1993) Identification of a peptide which binds to the carbohydrate-specific monoclonal antibody B3. Gene 128, 43–49.

[98] Oldenburg, K.R., Loganathan, D., Goldstein, I.J., Schultz, P.G. and Gallop, M.A. (1992) Peptide ligands for a sugar-binding protein isolated from a random peptide library. Proc. Natl. Acad. Sci. USA 89, 5393–5397.

[99] O'Neil, K.T., Hoess, R.H., Jackson, S.A., Ramachandran, N.S., Mousa, S.A. and DeGrado, W.F. (1992) Identification of novel peptide antagonists for GPIIb/IIIa from a conformationally constrained phage peptide library. Proteins 14, 509–515.

[100] Scott, J.K., Loganathan, D., Easley, R.B., Gong, X. and Goldstein, I.J. (1992) A family of concanavalin A-binding peptides from a hexapeptide epitope library. Proc. Natl. Acad. Sci. USA 89, 5398–5402.

[101] Scott, J.K. (1992) Discovering peptide ligands using epitope libraries. Trends Biochem. Sci. 17, 241–245.

[102] Smith, G.P. and Scott, J.K. (1993) Libraries of peptides and proteins displayed on filamentous phage. Methods Enzymol. 217, 228–257.

[103] Zhong, G., Berry, J.D. and Choukri, S. (1997) Mapping epitopes of neutralizing monoclonal antibodies using phage random peptide libraries. J. Ind. Microbiol. Biotechnol. 19, 71–76.

[104] Hutchins, W.A., Kieber-Emmons, T., Carlone, G.M. and Westerink, M.A. (1999) Human immune response to a peptide mimic of *Neisseria meningitidis* serogroup C in hu-PBMC-SCID mice. Hybridoma 18, 121–129.

[105] Azmi, F.H., Lucas, A.H., Raff, H.V. and Granoff, D.M. (1994) Variable region sequences and idiotypic expression of a protective human immunoglobulin M antibody to capsular polysaccharides of *Neisseria meningitidis* group B and *Escherichia coli* K1. Infect. Immun. 62, 1776–1786.

[106] Adderson, E.E., Shackelford, P.G., Quinn, A. and Carroll, W.L. (1991) Restricted Ig H chain V gene usage in the human antibody response to *Haemophilus influenzae* type b capsular polysaccharide. J. Immunol. 147, 1667–1674.

[107] Agadjanyan, M., Luo, P., Westerink, M.A., Carey, L.A., Hutchins, W., Steplewski, Z., Weiner, D.B. and Kieber-Emmons, T. (1997) Peptide mimicry of carbohydrate epitopes on human immunodeficiency virus. Nat. Biotechnol. 15, 547–551.

[108] Orlandi, R., Menard, S., Colnaghi, M.I., Boyer, C.M. and Felici, F. (1994) Antigenic and immunogenic mimicry of the HER2/neu oncoprotein by phage-displayed peptides. Eur. J. Immunol. 24, 2868–2873.

[109] Meola, A., Delmastro, P., Monaci, P., Luzzago, A., Nicosia, A., Felici, F., Cortese, R. and Galfre, G. (1995) Derivation of vaccines from mimotopes. Immunologic properties of human hepatitis B virus surface antigen mimotopes displayed on filamentous phage. J. Immunol. 154, 3162–3172.

[110] Lowell, G.H., Ballou, W.R., Smith, L.F., Zollinger, W.D. and Hockmeyer, W.T. (1989) Proteosome-hydrophobic 'foot' malaria peptide vaccines for *Plasmodium falciparum* and *P. vivax*. Trans. R. Soc. Trop. Med. Hyg. 83, 101–102.

[111] Valadon, P., Nussbaum, G., Oh, J. and Scharff, M.D. (1998) Aspects of antigen mimicry revealed by immunization with a peptide mimetic of *Cryptococcus neoformans* polysaccharide. J. Immunol. 161, 1829–1836.

[112] Young, A.C., Valadon, P., Casadevall, A., Scharff, M.D. and Sacchettini, J.C. (1997) The three-dimensional structures of a polysaccharide binding antibody to *Cryptococcus neoformans* and its complex with a peptide from a phage display library: implications for the identification of peptide mimotopes. J. Mol. Biol. 274, 622–634.

[113] Butcher, D.J. and Moe, G.R. (1996) Role of hydrophobic interactions and desolvation in determining the structural properties of a model alpha beta peptide. Proc. Natl. Acad. Sci. USA 93, 1135–1140.

[114] Butcher, D.J., Bruch, M.D. and Moe, G.R. (1995) Design and characterization of a model alpha beta peptide. Biopolymers 36, 109–120.

[115] Sieber, V. and Moe, G.R. (1996) Interactions contributing to the formation of a beta-hairpin-like structure in a small peptide. Biochemistry 35, 181–188.

[116] Tettelin, H., Dougherty, B.A., Hickey, E.K., White, O., Mason, T., Utterback, T., Smith, H.O., Venter, J.C. and Fraser, C.M. (1998) Sequencing of *Neisseria meningitidis* serogroup B strain MC58. In: Abstracts of the Eleventh International Pathogenic Neisseria Conference (Nassif, X., Quentin-Millet, M.-J. and Taha, M.-K., Eds.), p. 41. Editions E.D.K., Paris, France.

[117] Parkhill, J., Spratt, B., Achtman, M., Churcher, C., Basham, D., Chillingworth, T., Devlin, K., Feltwell, T., Hamlin, N., Holroyd, S., Jagels, K., Leather, S., Moule, S., Mungall, K., Skelton, J., Whitehead, S. and Barrell, B. (1998) Genome sequencing of *Neisseria meningitidis* serogroup A strain Z2491. In: Abstracts of the Eleventh International Pathogenic Neisseria Conference (Nassif, X., Quentin-Millet, M.-J. and Taha, M.-K., Eds.), p. 40. Editions E.D.K., Paris, France.

[118] Lewis, L.A., Hartman, K., Vaughn, J., Clifton, S.W., Lin, S.P., Song, L., Zhu, H., Kenton, S., White, J., Reece, A., Roe, B.A. and Dyer, D.W. (1998) The *Neisseria gonorrhoeae* genome project: an update. In: Abstracts of the Eleventh International Pathogenic Neisseria Conference (Nassif, X., Quentin-Millet, M.-J. and Taha, M.-K., Eds.), p. 42. Editions E.D.K., Paris, France.

[119] Martens, C.L., Cwirla, S.E., Lee, R.Y., Whitehorn, E., Chen, E.Y., Bakker, A., Martin, E.L., Wagstrom, C., Gopalan, P. and Smith, C.W. et al. (1995) Peptides which bind to E-selectin and block neutrophil adhesion. J. Biol. Chem. 270, 21129–21136.

[120] Charalambous, B.M. and Feavers, I.M. (1998) Identification and characterization of consensus peptides that cross-react with anti-*Neisseria meningitidis* LOS 3, 7, 9 monoclonal antibodies. In: Abstracts of the Eleventh International Pathogenic Neisseria Conference (Nassif, X., Quentin-Millet, M.-J. and Taha, M.-K., Eds.), p. 186. Editions E.D.K., Paris, France.

[121] Kieber-Emmons, T., Luo, P., Qiu, J., Agadjanyan, M., Carey, L., Hutchins, W., Westerink, M.A. and Steplewski, Z. (1997) Peptide mimicry of adenocarcinoma-associated carbohydrate antigens. Hybridoma 16, 3–10.